

Mathew L. Warman et al.
REGULATOR GENE SYSTEM USEFUL FOR THE
DIAGNOSIS AND THERAPY OF OSTEOPOROSIS
ATTORNEY DOCKET NO:38464-0004
Figure 1

Figure 1

-	Gene symbol	Gene name
5	OSBP	Oxysterol-binding protein
	NFKB3 (p65)	nuclearfactor of kappa light polypeptide gene enhancer B-cells 3
10	CAPN1	Calpain, large polypeptide L1
	CCND1	Cyclin D1
15	EFEMP2*	EGF-containing fibulin-like extracellular matrix protein 2
5	FOSL1	FOS-like antigen-1
5 5 1 1 1 20	PLCB3	Phospholipase C, beta 3 (phosphatidylinositol-specific)
=20 4	PPP1CA	Protein phosphatase-1, catalytic subunit, alpha isoform
1	VEGFB	Vascular endothelial growth factor B
25	ESRRA, ERR1	estrogen-related receptor alpha
11 -	CTSW	Cathepsin W
] [30	GALN*	Galanin
[=D()	LRP5, LRP7, LR3*	Low density lipoprotein receptor-related protein-5
	CBP2 .	Collagen-binding protein 2 (colligen 2)
35	TCIRG1	T-cell immune regulator 1
	LTBP3	Latent transforming growth factor-beta binding protein-3
40	FGF19*	Fibroblast growth factor 19 (FGF19)
40	Delta5-desaturase*	Delta5-desaturase

Figure 2

5 GCCAACCGCCGGGACGTACGGCTGGTGGACGCCGGCGGAGTCAAGCTGGAGTC CACCATCGTGGTCAGCGGCCTGGAGGATGCGGCCGCAGTGGACTTCCAGTTTTC CAAGGGAGCCGTGTACTGGACAGACGTGAGCGAGGAGGCCATCAAGCAGACCT ACCTGAACCAGACGGGGCCGCCGTGCAGAACGTGGTCATCTCCGGCCTGGTCT 10 CAGAGACCAACCGCATCGAGGTGGCCAACCTCAATGGCACATCCCGGAAGGTGC TCTTCTGGCAGGACCTTGACCAGCCTAGGGCCATCGCCTTGGACCCCGCTCACG GGTACATGTACTGGACAGACTGGGGTGAGACGCCCCGGATTGAGCGGGCAGGG ATGGATGGCAGCACCCGGAAGATCATTGTGGACTCGGACATTTACTGGCCCAAT GGACTGACCATCGACCTGGAGGAGCAGAAGCTCTACTGGGCTGACGCCAAGCTC 15 AGCTTCATCCACCGTGCCAACCTGGACGGCTCGTTCCGGCAGAAGGTGGTGGAG GGCAGCCTGACGCACCCCTTCGCCCTGACGCTCTCCGGGGACACTCTGTACTGG ACAGACTGGCAGACCCGCTCCATCCATGCCTGCAACAAGCGCACTGGGGGGAAG AGGAAGGAGATCCTGAGTGCCCTCTACTCACCCATGGACATCCAGGTGCTGAGC J 20 CAGGAGCGGCAGCCTTTCTTCCACACTCGCTGTGAGGAGGACAATGGCGGCTGC ıD TCCCACCTGTGCCTGTCCCCAAGCGAGCCTTTCTACACATGCGCCTGCCCCA Į. CGGGTGTGCAGCTGCAGGACAACGGCAGGACGTGTAAGGCAGGAGCCGAGGAG -GTGCTGCTGCCCGGCGGACGGACCTACGGAGGATCTCGCTGGACACGCCG 25 25 GACTTCACCGACATCGTGCTGCAGGTGGACGACATCCGGCACGCCATTGCCATC GACTACGACCCGCTAGAGGGCTATGTCTACTGGACAGATGACGAGGTGCGGGCC ATCCGCAGGGCGTACCTGGACGGGTCTGGGGCGCAGACGCTGGTCAACACCGAG ATCAACGACCCCGATGGCATCGCGGTCGACTGGGTGGCCCGAAACCTCTACTGG ACCGACACGGGCACGGACCGCATCGAGGTGACGCGCCTCAACGGCACCTCCCGC |= AAGATCCTGGTGTCGGAGGACCTGGACGAGCCCCGAGCCATCGCACTGCACCCC 30 GTGATGGCCTCATGTACTGGACAGACTGGGGAGAGAACCCTAAAATCGAGTGT CCCAACGCCTGGCCCTGGACCTGCAGGAGGGGAAGCTCTACTGGGGAGACGCC AAGACAGACAAGATCGAGGTGATCAATGTTGATGGGACGAAGAGGCGGACCCT CCTGGAGGACAAGCTCCCGCACATTTTCGGGTTCACGCTGCTGGGGGACTTCAT 35 CTACTGGACTGACTGCAGCGCCGCAGCATCGAGCGGGTGCACAAGGTCAAGGC CAGCCGGGACGTCATCATTGACCAGCTGCCCGACCTGATGGGGCTCAAAGCTGT GAATGTGGCCAAGGTCGTCGGAACCAACCCGTGTGCGGACAGGAACGGGGGGT GCAGCCACCTGTGCTTCTTCACACCCCACGCAACCCGGTGTGGCTGCCCCATCG GCCTGGAGCTGAGTGACATGAAGACCTGCATCGTGCCTGAGGCCTTCTTGG 40 TCTTCACCAGCAGAGCCGCCATCCACAGGATCTCCCTCGAGACCAATAACAACG ACGTGGCCATCCCGCTCACGGGCGTCAAGGAGGCCTCAGCCCTGGACTTTGATG TGTCCAACAACCACATCTACTGGACAGACGTCAGCCTGAAGACCATCAGCCGCG CCTTCATGAACGGGAGCTCGGTGGAGCACGTGGTGGAGTTTGGCCTTGACTACC CCGAGGGCATGGCCGTTGACTGGATGGGCAAGAACCTCTACTGGGCCGACACTG GGACCAACAGAATCGAAGTGGCGCGGCTGGACGGCAGTTCCGGCAAGTCCTC . 45 GTGTGGAGGGACTTGGACAACCCGAGGTCGCTGGCCCTGGATCCCACCAAGGGC TACATCTACTGGACCGAGTGGGGCGGCAAGCCGAGGATCGTGCGGGCCTTCATG

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Figure 2 (Page 2 of 3)

GACGGGACCAACTGCATGACGCTGGTGGACAAGGTGGGCCGGGCCAACGACCT GATCGAGTCGTCCAACATGCTGGGTCAGGAGCGGGTCGTGATTGCCGACGATCT CCCGCACCCGTTCGGTCTGACGCAGTACAGCGATTATATCTACTGGACAGACTG 5 GAATCTGCACAGCATTGAGCGGCCGACAAGACTAGCGGCCGGAACCGCACCCT CATCCAGGGCCACCTGGACTTCGTGATGGACATCCTGGTGTTCCACTCCTCCCGC CAGGATGGCCTCAATGACTGTATGCACAACAACGGGCAGTGTGGGCAGCTGTGC CTTGCCATCCCGGCGGCCACCGCTGCGCTGCGCCTCACACTACACCCTGGAC CCCAGCAGCCGCAACTGCAGCCCGCCCACCACCTTCTTGCTGTTCAGCCAGAAA TCTGCCATCAGTCGGATGATCCCGGACGACCAGCACAGCCCGGATCTCATCCTG 10 CCCCTGCATGGACTGAGGAACGTCAAAGCCATCGACTATGACCCACTGGACAAG TTCATCTACTGGGTGGATGGGCCCAGAACATCAAGCGAGCCAAGGACGACGG GACCCAGCCTTTGTTTTGACCTCTCTGAGCCAAGGCCAAAACCCAGACAGGCA GCCCCACGACCTCAGCATCGACATCTACAGCCGGACACTGTTCTGGACGTGCGA GGCCACCAATACCATCAACGTCCACAGGCTGAGCGGGGAAGCCATGGGGGTGG 15 TGCTGCGTGGGGACCGCGACAAGCCCAGGGCCATCGTCGTCAACGCGGAGCGA GGGTACCTGTACTTCACCAACATGCAGGACCGGGCAGCCAAGATCGAACGCGCA GCCTGGACGCACCGAGCGCGAGGTCCTCTTCACCACCGGCCTCATCCGCCCT GTGGCCTGGTGGTGGACACACACTGGGCAAGCTGTTCTGGGTGGACGCGGAC CTGAAGCGCATTGAGAGCTGTGACCTGTCAGGGGCCAACCGCCTGACCCTGGAG 20 GACGCCAACATCGTGCAGCCTCTGGGCCTGACCATCCTTGGCAAGCATCTCTAC TGGATCGACCGCCAGCAGCAGATGATCGAGCGTGTGGAGAAGACCACCGGGGA CAAGCGGACTCGCATCCAGGGCCGTGTCGCCCACCTCACTGGCATCCATGCAGT GGAGGAAGTCAGCCTGGAGGAGTTCTCAGCCCACCCATGTGCCCGTGACAATGG **I**125 TGGCTGCTCCCACATCTGTATTGCCAAGGGTGATGGGACACCACGGTGCTCATG CCCAGTCCACCTCGTGCTCCAGAACCTGCTGACCTGTGGAGAGCCGCCCAC CTGCTCCCGGACCAGTTTGCATGTGCCACAGGGGAGATCGACTGTATCCCCGG GGCCTGGCGCTGTGACGGCTTTCCCGAGTGCGATGACCAGAGCGACGAGGAGGG CTGCCCGTGTGCTCCGCCGCCCAGTTCCCCTGCGCGCGGGGTCAGTGTGTGGA **5**30 CCTGCGCCTGCGCTGCGACGGCGAGGCAGACTGTCAGGACCGCTCAGACGAGGC GGACTGTGACGCCATCTGCCTGCCCAACCAGTTCCGGTGTGCGAGCGGCCAGTG TGTCCTCATCAAACAGCAGTGCGACTCCTTCCCCGACTGTATCGACGGCTCCGA CGAGCTCATGTGTGAAATCACCAAGCCGCCCTCAGACGACAGCCCGGCCCACAG 35 GTCTATTTTGTGTGCCAGCGCGTGGTGTGCCAGCGCTATGCGGGGGCCAACGGG CCCTTCCCGCACGAGTATGTCAGCGGGACCCCGCACGTGCCCCTCAATTTCATA GCCCCGGGCGTTCCCAGCATGGCCCCTTCACAGGCATCGCATGCGGAAAGTCC CGGAACCACGTCACAGGGCCTCGTCCAGCAGCTCGTCCAGCACGAAGGCCACG 40 TACAACATGGACATGTTCTACTCTTCAAACATTCCGGCCACTGCGAGACCGTAC AGGCCCTACATCATTCGAGGAATGGCGCCCCGACGACGCCCTGCAGCACCGAC GTGTGTGACAGCGACTACAGCGCCAGCCGCTGGAAGGCCAGCAAGTACTACCTG 45 TACCTGTCGGCGGAGGACAGCTGCCCGCCCTCGCCGCCACCGAGAGGAGCTAC TTCCATCTCTTCCCGCCCCCTCCGTCCCC CTGCACGGACTCATCCTGACCTCGGCCGGCCACTCTGGCTTCTCTGTGCCCCTG TAAATAGTTTTAAATATGAACAAAGAAAAAAATATATTTTATGATTTAAAAAAAT

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 ${\bf AAATATAATTGGGATTTTAAAAACATGAGAAATGTGAACTGTGATGGGGTGGGCAGGGCTGGGAGAACTTTGTA}$

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Figure 3

MEAAPPGPPWPLLLLLLLLLALCGCPAPAAASPLLLFANRRDVRLVDAGGVKLESTI VVSGLEDAAAVDFQFSKGAVYWTDVSEEAIKQTYLNQTGAAVQNVVISGLVSPDGL ACDWVGKKLYWTDSETNRIEVANLNGTSRKVLFWQDLDQPRAIALDPAHGYMYW TDWGETPRIERAGMDGSTRKIIVDSDIYWPNGLTIDLEEQKLYWADAKLSFIHRANL DGSFROKVVEGSLTHPFALTLSGDTLYWTDWQTRSIHACNKRTGGKRKEILSALYSP MDIQVLSQERQPFFHTRCEEDNGGCSHLCLLSPSEPFYTCACPTGVQLQDNGRTCKA GAEEVLLLARRTDLRRISLDTPDFTDIVLQVDDIRHAIAIDYDPLEGYVYWTDDEVR AIRRAYLDGSGAOTLVNTEINDPDGIAVDWVARNLYWTDTGTDRIEVTRLNGTSRK 10 ILVSEDLDEPRAIALHPVMGLMYWTDWGENPKIECANLDGQERRVLVNASLGWPN GLALDLQEGKLYWGDAKTDKIEVINVDGTKRRTLLEDKLPHIFGFTLLGDFIYWTD WORRSIERVHKVKASRDVIIDQLPDLMGLKAVNVAKVVGTNPCADRNGGCSHLCFF TPHATRCGCPIGLELLSDMKTCIVPEAFLVFTSRAAIHRISLETNNNDVAIPLTGVKE []15 ASALDFDVSNNHIYWTDVSLKTISRAFMNGSSVEHVVEFGLDYPEGMAVDWMGKN LYWADTGTNRIEVARLDGQFRQVLVWRDLDNPRSLALDPTKGYTYWTEWGGKPRI VRAFMDGTNCMTLVDKVGRANDLTIDYADQRLYWTDLDTNMIESSNMLGQERVV IADDLPHPFGLTQYSDYIYWTDWNLHSIERADKTSGRNRTLIQGHLDFVMDILVFHS SRQDGLNDCMHNNGQCGQLCLAIPGGHRCGCASHYTLDPSSRNCSPPTTFLLFSQKS AISRMIPDDQHSPDLILPLHGLRNVKAIDYDPLDKFIYWVDGRQNIKRAKDDGTQPF VLTSLSOGONPDROPHDLSIDIYSRTLFWTCEATNTINVHRLSGEAMGVVLRGDRDK PRAIVVNAERGYLYFTNMQDRAAKIERAALDGTEREVLFTTGLIRPVALVVDNTLG KLFWVDADLKRIESCDLSGANRLTLEDANIVQPLGLTILGKHLYWIDRQQQMIERVE KTTGDKRTRIQGRVAHLTGIHAVEEVSLEEFSAHPCARDNGGCSHICIAKGDGTPRC 25 SCPVHLVLLONLLTCGEPPTCSPDQFACATGEIDCIPGAWRCDGFPECDDQSDEEGC PVCSAAQFPCARGQCVDLRLRCDGEADCQDRSDEADCDAICLPNQFRCASGQCVLI KOOCDSFPDCIDGSDELMCEITKPPSDDSPAHSSAIGPVIGIILSLFVMGGVYFVCQRV VCQRYAGANGPFPHEYVSGTPHVPLNFIAPGGSQHGPFTGIACGKSMMSSVSLMGG RGGVPLYDRNHVTGASSSSSSSTKATLYPPILNPPPSPATDPSLYNMDMFYSSNIPAT ARPYRPYIIRGMAPPTTPCSTDVCDSDYSASRWKASKYYLDLNSDSDPYPPPPTPHSQ 30 YLSAEDSCPPSPATERSYFHLFPPPPSPCTDSS

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Figure 4

Figure 4

Source	Nucleotide change	Protein change	Putative effect
OPS 88	G29A	Trp10stop	Truncation
Control	A459G	Pro153Pro	No change
Control	InsCTG33	Insert Leu	Alters signal
		at residue 12	peptide
OPS 78	GACCTACG	AspLeuSer	Unknown
	1051-1058	351-353	
	ACCCTACA	ThrLeuLys	
OPS 59	C1282T	Arg428stop	Truncation
OPS 53	G1253T	Glu485stop	Truncation
OPS 23	delG1467	Frameshift	Truncation
OPS 82	G1481A	Arg494Gln	Unknown
OPS 2	C1708T	Arg570Trp	Unknown
OPS 2 OPS 72 OPS 45	G1999A	Val677Met	Unknown
OPS 45	insT2150	Frameshift	Truncation
₫ OPS 41	G2202A	Trp734stop	Unknown
Control	C2220T	Asn740Asn	Unknown
OPS 92 OPS 13	delG2305	Frameshift	Truncation
OPS13	C2557T	Gln853stop	Truncation
OPS 7	delA3804	Frameshift	Truncation
OPS 53	C3989T	Ala1330Leu	Unknown
DPS 72	C3989T .	Ala1330Leu	Unknown
[≟] Control	G4416T	Leu1472Leu	No change

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Figure 5

NAME	SEQUENCE	LOCATION	PRODUCT SIZE
LRGEN1F	5'-TTG CTG CCC TAG ACT TAG CC-3'	-119	406
LRGEN1R	5'-CCA AGT CGC TTC CGA GAC-3'	+106	
LRGEN2F	5'-CAT CCC AGG GCT GTG TAT CT-3'	-65	543
LRGEN2R	5'-ACT TGG GCT CAT GCA AAT TC-3'	+81	1
LRGEN3F1	5'-CCG ATG GGT GAG ATT TTA GG-3'	-118	329
LRGEN3R1	5'-CGT GGG TAC CTA CCG GAA C-3'	+16	
LRGEN4NF	5'-TAA TTG GGT CAG CAA TG-3'	-72	277
LRGEN4NR	5'-GCA CTC ACA GAA AGG CTG-3'	+8	
LRGEN5NF	5'-AGT GAC GGT CCT CTT CTG GA-3'	-51	302
LRGEN5NR	5'-CAA GTG GAT CAT TTC GAA CG-3'	+120	302
LRGEN6F	5'-TGG CTG AGT ATT TCC CTT GC-3'	-95	577
LRGENOF LRGEN6R	5'-CCA GAA TGA CAG GTC CAG GT-3'	+85	
	5'-TGC TTC TTC TCC AGC CTC AT-3'	-14	302
LRGEN7F1	5'-ATG TGG CCA AAT AGC AGA GC-3'	+116	302
LRGEN7R1	5'-GCA TTG AAC CCG TCT TGT TT-3'	-109	426
LRGEN8F	5'-GGC ACC TGA GCT CAA CAC TT-3'	+100	420
LRGEN8R	5'-TGC TGG GCT GTT GT GTT TA-3'	-47	407
LRGEN9F	5'-CTT TGA GGC AGG AAC AGA GG-3'	+70	1407
LRGEN9R	5'-AGC GAA ACT CCG TCT CAA AA-3'	-79	417
LRGEN10F	5'-GCT CTA ATC ACT GAG GGC CA-3'	+110	111
LRGEN10R	5'-GAG GGC TGA GCT GAA GAG GT-3'	-105	398
LRGEN11F1 LRGEN11R1	5'-CAG GTT GGG GAA CTT GCA G-3'	+108	370
LRGEN11R1	5'-ATT CAT GTG GTC GCT AGG CT-3'	-113	479
LRGEN12F	5'-GAA GCT CCT TTC AGC GTC AG-3'	+40	1475
LRGEN12R LRGEN13F	5'-CCA GCT CCT CTG TGG CTT AC-3'	-57	352
LRGEN13F	5'-TCC TCC CTC TGC TAA GGA CA-3'	+95	332
	5'-CAG ÁGC TCT CCA GCC AGT G-3'	-149	440
LRGEN14F	5'-CTG TGA GAG GCT GGC ATT C-3'	+82	1440
LRGEN14R LRGEN15NF	5'-ATG TGA CCT GTC AGC CTC G-3'	-131	415
		+83	1113
LRGEN15NR	5'-TGC TGC CAT TAC TGA CAA TGA-3' 5'-TCT GTC CTC CCA AGC TGA GT-3'	-76	374
LRGEN16F		+88	374
LRGEN16R	5'-CAC ACA GGA TCT TGC ACT GG-3'	-92	321
LRGEN17F	5'-CAT GAG TTC TCA TTT GGC CC-3'	+103	321
LRGEN17R	5'-GCC ACA GGG ACT GTG ATT TT-3'		423
LRGEN18F	5'-CAA CTT CTG CTT TGA AGC CC-3'	-88	742
LRGEN18R	5'-CAG AGC CCC TAC TCC TGT GA-3'	+98	269
LRGEN19F	5'-CCA GAC CTT GGT TGC TGT G-3'	81	209
LRGEN19R	5'-CGT CTC CTC CCC TAA ACT CC-3'	+77	210
LRGEN20NF	5'-ATG TTG GCC ACC TCT TTC TG-3'	-34	310
LRGEN20NR	5'-CTG CCT CCT CCA GAT CAT TC-3'	+39	272
LRGEN21F	5'-GAG TCT CGT GGG TAG TGG GA-3'	-102	373

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LRGEN21R	5'AGA AAG CAA GCA TGC CTC AG-3'	+131	
LRGEN22F	5'-AGC CCT CTC TGC AAG GAA AG-3'	-96	305
LRGEN22R	5'-GCC CAC TAG CAC CCA GAA TA-3'	+111	
LRGEN23F	5'-GAC AGG CCT TTC CCG TTC-3'	-95	650
LRGEN23R	5'-CAG GAG GAC TCT CAT GGT GG-3'	+106	
LRCOD1F	5'-TTC GTC ATG GGT GGT GTC TA-3'	4192	416
LRCOD1R	5'-TTC CTC GAA TGA TGT AGG GC-3'	4607	
LRCOD2F	5'-ACC TGG ACT TCG TGA TGG AC-3'	2654	466
LRCOD2R	5'-CAG AAC AGT GTC CGG CTG TA-3'	3119	
LRCOD3F	5'-CCA TGG AGC CCG AGT GAG-3'	-50	504
LRCOD3R	5'-GTC AAG GTC CTG CCA GAA GA-3'	453	
LRCOD4F	5'-GGG CAA GAA GCT GTA CTG GA-3'	354	500
LRCOD4R	5'-TGG ATG TCC ATG GGT GAG TA-3'	853	
LRCOD5F	5'-CAG ACC CGC TCC ATC CAT-3'	767	484
LRCOD5R	5'-TCG TTG ATC TCG GTG TTG AC-3'	1250	
LRCOD6F	5'-ATC GAC TAC GAC CCG CTA GA-3'	1132	546
LRCOD6R	5'-GTA GAT GAA GTC CCC CAG CA-3'	1677	
LRCOD7F	5'-GCC AAG ACA GAC AAG ATC GAG-	1564	505
÷ .	3'	1	
LRCOD7R	5'-TGT GGT TGT TGG ACA CAT CA-3'	2068	
LRCOD8F	5'-CAC AGG ATC TCC CTC GAG AC-3'	1966	522
LRCOD8R	5'-CTC GAT CAT GTT GGT GTC CA-3'	2487	
LRCOD9F	5'-CAG CCC TTT GTT TTG ACC TC-3'	3025	484
LRCOD9R	5-TCC AGT AGA GAT GCT TGC CA-3'	3508	
LRCOD10F	5'-AAG CGC ATT GAG AGC TGT G-3'	3400	480
LRCOD10R	5'-CTC CTC GTC GCT CTG GTC-3'	3879	
LRCOD11F	5'-CAC AGG GGA GAT CGA CTG TAT-	3801	480
*	3'		
LRCOD11R	5'-ACA TAC TCG TGC GGG AAG G-3'	4280	
LRCOD12F	5'-GTC CAG CAG CTC GTC CAG-3'	4446	567
LRCOD12R	5'-TAC AAA GTT CTC CCA GCC CT-3'	5012	
LRCOD13F	5'-TCA TGG ACG GGA CCA ACT-3'	2369	431
LRCOD13R	5'-GGT GTA GTG TGA GGC GCA G-3'	2799	

Figure 6

BMSR Construct Information

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- 5 The following BSMR expression constructs have been constructed using the pcDNA3 expression vector for use in following BSMR expression, function, and other biological (e.g. ligand and downstream signaling) interactions.
- 1. A full length wild type construct extending from primers LRCOD3F to LRCOD12R.

Sequence encoding a FLAG antibody epitope (GAC TAC AAG GAC GAC GAT GAC AAG) was inserted into the wild-type construct immediately downstream of nucleotide 165 (relative to the "A" in the ATG translation start site). This construct expresses a BSMR protein which has a FLAG epitope between wild type BSMR amino acid residues 55 and 56.

This was accomplished using the following primer sequences and a Quickchange reaction:

LRPFLAGF: 5'-GAC TAC AAG GAC GAC GAT GAC AAG ACC ATC GTG GTC AGC GGC CTG-3'

LRPFLAGR:5'-CTT GTC ATC GTC GTC CTT GTA GGA CTC CAG CTT GAC TCC GCC-3'

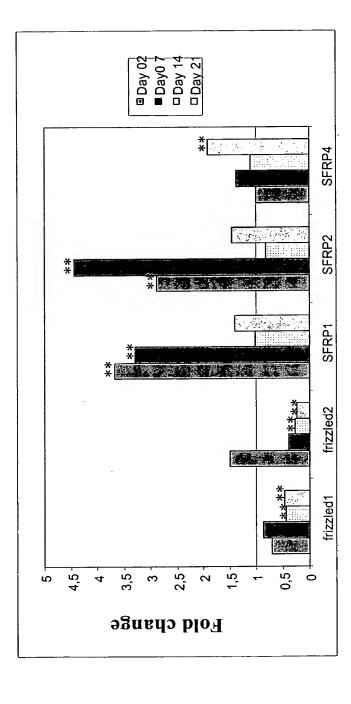
Sequence encoding a MYC antibody epitope (GAG CAG AAG CTG ATA TCC GAG GAG GAC CTG) was inserted immediately uptream of the stop codon after residue 4845 (relative to the "A" in the ATG translation start site). The construct expresses a BSMR protein which has a MYC epitope at the end of the wild type BSMR polypeptide.

This was accomplished using the following primer sequences and a Quickchange reaction:

LRPMYCF: 5'-GAG CAG AAG CTG ATA TCC GAG GAG GAC CTG TGA CCT CGG CCG GGC-3'

LRPMYCR: 5'-CAG GTC CTC GGA TAT CAG CTT CTG CTC GGA TGA GTC CGT GCA-3'

A expresssion construct containing both the FLAG and MYC antibody epitopes at the aforementioned sites has also been produced.



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■ BMSC_R2/R5 □ Trabec_R3/R5 ☐ Trabec_R2/R5 ■ BMSC_R3/R5 SFRP1 frizzled7 frizzled4 frizzled3 frizzled1 wnt10-like wnt13/2B wnt5a 1,00 4,50 3,50 3,00 2,50 2,00 1,50 5,50 5,00 Fold change

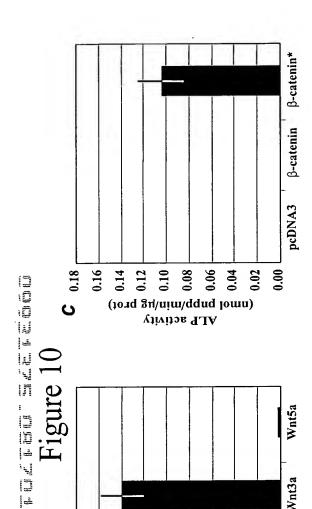
Figure 8

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Figure 9

Figure 9

Cene name	CCC12/BMP2 (4 days)	C3H10T1/2/BNP2+SHH (4 days)	STZ/BMP2 (4days)	MC3T3-E1 (3days)
Frizzled-1	2.25 x	N.	2.62x	2.11x
Frizzled4	NR	4.37x	N.	NR
SFRP2/SARP1	8.10 x	0.5x (0.4x with SHHalone)	8.54x	3.61x (0.09 with TGPDeta)

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9.0

(nmol papp/min/µg prot)

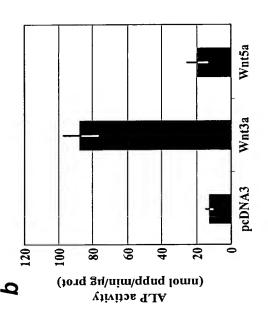
ALP activity

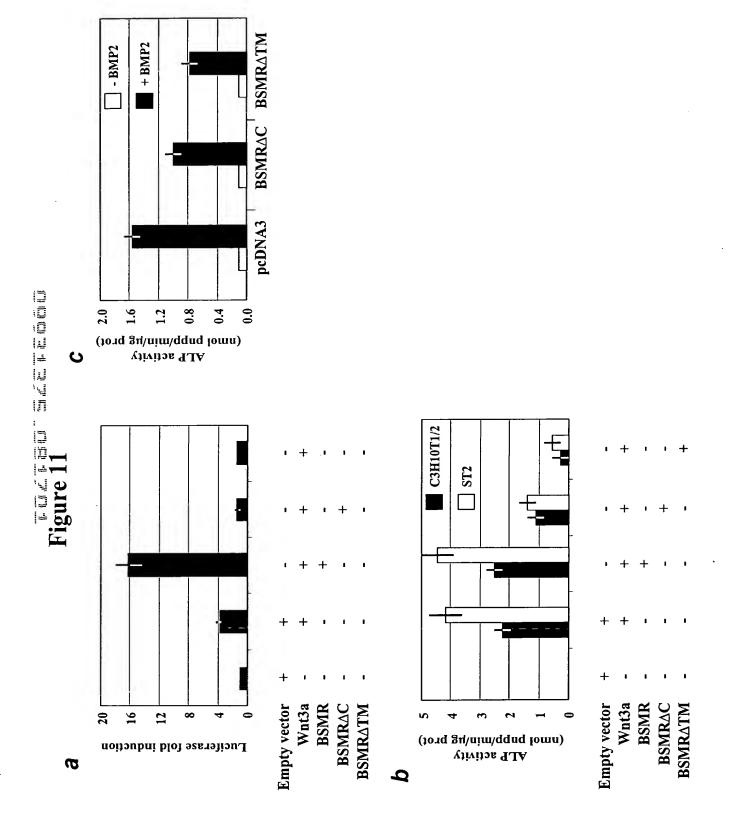
1.4 1.2 1.0

B

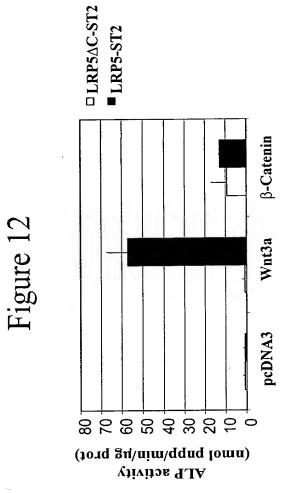
Wnt3a

pcDNA3









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Figure 13

Figure 13

RVRLASHLRKLRK

RLTRKRGLKLA

CRAKRNNFKSA

LKWKS

KIRVKAGETQKKVIFCSREKVSHL

FIPLKPTVKMLERSNHVSRTEVSSNHV

DKGMAPALRHLYKELMGPWN

DALKLAIDNALSIT